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Published in:
Organohalogen Comp

Publication date:
2003

Document Version
Publisher's PDF, also known as Version of record

PCBs AND ORGANOCHLORINE PESTICIDE CONCENTRATIONS IN A FAROE ISLAND 14-YEAR OLD COHORT: MEASUREMENT USING NEW METHODOLOGY AND EVALUATION OF CORRELATIONS AND PATTERNS

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Introduction

Polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides are manmade chemicals that are pervasive in our environment [1]. Although originally produced for a variety of commercial applications such as heat transfer liquids or dielectric fluids, PCBs remain persistent in the environment either from use or careless disposal practices [1]. Similarly, the extensive use of OC pesticides in the mid-20th century for vector-borne disease control and residential and agricultural applications has left a reservoir of these chemicals in our environment [2]. Despite the obvious benefits of both PCBs and OC pesticides in the mid- to late-20th century, their impact on the environment and public health has been substantial.

Most OC pesticides and PCBs are considered persistent organic pollutants (POPs) in the environment. These POPs have long environmental half-lives and tend to bioaccumulate in humans and other animals, and thus biomagnify up to 70,000 times in the food chain [3,4], particularly in sustenance foods such as blubber and fatty fish.

Residents of the Faroe Islands, a Nordic fishing community comprised of 18 islands located in the North Atlantic between Scotland and Iceland, are a homogeneous and stable population that is expected to have higher exposures to PCBs and other persistent environmental pollutants due to their large consumption of pilot whale blubber [5]. In 1986-87, a birth cohort of approximately 1000 children was established in the Faroe Islands where whale and fish constitute an average of 55% of all dinner meals [5].

We measured PCBs and OC pesticides in 876 serum samples collected from this Faroese birth cohort at age 14 years. We used a novel method combining accelerated solvent extraction and gel permeation chromatography purification coupled with isotope dilution-gas chromatography-high resolution mass spectrometry. We report PCB and OC pesticide patterns and intercorrelations in children of the Faroe Island cohorts. In addition, we evaluate the biological measurements in relation to two of the potential exposure sources: breastfeeding and blubber consumption.

Methods and Materials

Study Cohort

The Faroese cohort has been described previously [5]. Briefly, a birth cohort of 1022 children was established from 1986-87. Both prenatal and postnatal exposure data were collected. The data collection included questionnaires in which the demography of the participant, maternal diet during pregnancy, duration of breast feeding, and blubber consumption at age 7 and 14 were recorded. Maternal blood samples were taken before birth. In addition, umbilical cord blood and breast milk samples were obtained shortly after birth. Blood samples were also collected from the children at ages 7 and 14.

Sample Analysis
Serum samples (1 g) were weighed into 22-mL extraction cells that contained a frit and 3 g hydromatrix (Varian, Palo Alto, CA). The serum samples were spiked with labeled internal standards and allowed to equilibrate. The serum samples were frozen to -35 ºC over 3 hours in a commercial lyophilizer (Virtis Genesis,25 SQ EL, Gardiner, NY) and then dried under a 200 mT vacuum for 9 hours. The dried serum samples were allowed to warm to room temperature in a desiccator. The extraction cells were then fitted with an additional frit and topped with 4.5 g activated Florisil®. The Florisil® was topped with two frits then the extraction cells were capped and inverted. The inverted extraction cells were placed on the rack of an automated accelerated solvent extractor (ASE; Dionex, Sunnyvale, CA). On the ASE unit, each sample was heated for 5 min at 100 ºC and then subjected to a static extraction at 1500 psi for 5 min using 20% dichloromethane in hexane. The extraction cell was flushed with 60% volume into a centrifuge tube. The static extraction process was repeated. The final extract volume was around 30 mL. The sample volumes were reduced to approximately 200 µL using a RapidVap vacuum concentrator (LabConco, Kansas City, MO). The extracts were further purified as previously described [6] using high-resolution gel permeation chromatography (HR-GPC) on an Agilent 1100 high performance-liquid chromatrograph (HPLC; Agilent Technologies, Atlanta, GA), equipped with a Gilson Fraction Collector (Gilson Inc., Middleton, WI). The HR-GPC chromatographic separations were performed on a polystyrene-divinyl benzene stationary phase HPLC column (Plgel 10um, 100Ă, 300x7.5mm) from Polymer Laboratories Inc. (Amherst, MA). Dichloromethane was the mobile phase and was kept at 1mL/min flow rate. The fraction of solvent eluting between 8.1 and 12 minutes was collected, and reduced in volume to 200 µL under vacuum. The extract was spiked with recovery standard (^{13}C –TCDD in nonane) and allowed to concentrate at ambient temperature to the final volume (10µL) for HRMS quantification.

All samples were analyzed on a MAT 95 mass spectrometer (maximum acceleration voltage 5kV) equipped with a 6890 gas chromatography (GC; Agilent Technologies, Atlanta, GA). PCB and pesticide analysis was completed simultaneously with a single injection. The GC was operated in the splitless injection mode with a constant flow of 1mL/min of helium through a 30m x 0.25mm DB-5MS column with a 0.25µm film thickness. The injector was set at 290°C and transfer line was set at 270°C. The initial column temperature was set at 100°C; held for 0.6 minute; heated to 200°C at 25°C/min; held at 200°C for 5min; heated to 250°C at 4°C/min; and then heated to 320°C at 35°C/min and held at 320°C for 3min. All spectra were recorded with low energy (50eV) electron impact ionization with a resolution of 10,000 (10% valley definition). To achieve simultaneous analysis of PCB and pesticides, mass ions were optimized for each individual pesticide and PCB as described elsewhere [7].

All PCBs and pesticides in unknown samples, quality control materials, and procedural blanks were quantified from ^{13}C isotope dilution continuing calibration plots.

Statistical Analysis
All data were evaluated using SAS statistical software (SAS institute, Cary, NC). Pearson correlation analyses were used to determine intercorrelations of the PCBs and OC pesticides. An analysis of covariance (ANCOVA) was used to determine the least squares geometric means (LSGMs) of each variable tested while correcting for the other two covariates. The variables tested were short duration of breast-feeding (exclusive nursing up to 1 month and total nursing up to 2 months), blubber consumption at age 7 and/or 14, and sex. Differences among the LSGMs of each variable were considered significant if $p \leq 0.05$.

Results and Discussion
We evaluated the intecorrelations of the PCB congeners and pesticides detected in greater than 90% of the samples tested. In general, the most highly chlorinated PCBs, especially pentachloro and higher, were strongly intercorrelated, but showed poor correlations with the lower chlorinated PCBs. Interestingly, the PCBs of lower chlorination were highly intercorrelated with each other. This is in direct contrast to the observations of Gladen et al. [8] who reported no correlations for the lower molecular weight PCBs, albeit their analysis was on a data set generated from breast milk samples.

We also observed strong intercorrelations among the OC pesticides. Interestingly, with the exception of γ-HCCH, we found highly significant correlations among selected PCBs and the OC pesticides. For

example, we found that the commonly measured PCBs 153 and 180 were highly correlated with oxychlordane, *trans*-nonachlor, p,p'-DDE, and mirex ($0.65<r<0.94$), but not as strongly correlated with heptachlor epoxide, dieldrin, p,p'-DDT, and o,p'-DDT ($r<0.38$), although all correlations were statistically significant. However, the concentrations of mono-orthosubstituted PCBs 105 and 118 showed strong correlations that were similar among the same pesticides ($0.52<r<0.83$). Hexachlorobenzene and β-HCCH showed similar, more scattered correlations with all of the most prevalent PCBs ($r\sim0.3$). Although most studies have failed to show significant correlations between PCBs and OC pesticides, Glynn et al. [9] saw similar correlations for individual congeners.

The PCB patterns of the 12 most prevalently detected PCBs are shown in Figure 1. As shown, the PCB congener 153 was detected in the highest concentrations followed by PCBs 180, 138/158, and 187. This pattern was slightly different than that reported for Michigan fish eaters in which the highest concentrations were for PCB138 [10]. The patterns we found were similar whether the samples were separated by potential exposure sources (blubber consumption or breastfeeding); however, there was a significant sex interaction. Not surprisingly, males, who both nursed for a longer duration and consumed blubber as a child and adolescent, had the highest PCB. However, males, who consumed blubber or nursed only a short duration but not both, had similar PCB concentrations. Conversely, for females, blubber consumption was the single most significant contributor to their PCB concentrations. Although nursing did contribute to the elevated PCB concentrations, nursing alone did not have the same impact on PCB concentrations as did blubber consumption. We observed a similar pattern for p,p'-DDE concentrations in males and females. Males who nursed or consumed blubber at either age 7 or 14 but not both had similar concentrations of p,p'-DDE. However, females, who nursed a short duration, had similar or higher p,p'-DDE concentrations than did females who nursed a longer duration. Clearly, blubber consumption had a greater effect on p,p'-DDE levels than did nursing.

We evaluated the average concentrations of PCB congeners, both individually and as groupings based upon their intercorrelations, and their association with nursing, blubber consumption and sex. When summing all detectable PCB concentrations, we observed a strong concentration gradient based upon blubber consumption. Those children, who were known to have consumed blubber at ages 7 and 14, had higher total PCB concentrations (8,416 pg/g) than those who consumed blubber at age 7 or 14 but not both (6,867 pg/g)or who did not consume blubber at all (5,625 pg/g); however, none of the differences were significant. When the individual congeners were considered, the most highly chlorinated congeners that are prevalently found in humans such as 105, 118, 153, 138/58, 180, and 187 showed concentration gradients that were highly significant. For example, PCB 153 mean concentrations were 1924, 1313, and 875 pg/g for those who consumed blubber at 7 and 14, those who consumed it at only one age, and non-consumers, respectively (individual p values <0.001 and $p<0.001$ for trend).

When grouped into higher molecular weight PCBs (pentachloro and above) and lower molecular weight PCBs, the concentration gradient for blubber consumption was clearly apparent for the higher molecular weight PCBs but absent for the lower molecular weight ones. This suggests that exposure to the lower molecular weight PCBs may occur from a source other than blubber or one or more sources in addition to blubber that were not identified in this analysis.

The duration of nursing showed a similar trend. Children who nursed for a shorter duration had lower concentrations of the higher molecular weight PCBs than did children who nursed for a longer duration ($p<0.001$). Interestingly, the mono-orthosubstituted PCBs 105 and 118 were not associated with nursing duration ($p=0.66$ and $p=0.73$, respectively).

Conclusion

We successfully applied our new analytic methodology for the analysis of approximately 876 serum samples from 14-year olds in the Faroe birth cohort. We found a concentration gradient in the more highly chlorinated PCBs among children who consumed blubber at both age 7 and 14 years, those who consumed blubber at only one of those time points, and those who did not consume blubber at all. In addition, we observed a significant difference in PCB concentrations between those children that nursed a longer duration and those who only nursed a short duration; however, the association with nursing duration was not observed for the mono-orthosubstituted PCBs 105 and 118. We saw, also, a significant difference in

serum PCB concentrations between males and females. Interestingly, we found a strong interaction between sex and nursing duration. Nursing duration had a lesser impact on the PCB and p,p'-DDE levels in females than in males.

Acknowledgements

This research was supported in part by the National Institute of Environmental Health Sciences (ES09797). The contents of this paper are solely the responsibility of the authors and do not represent the official views of the NIEHS or NIH.

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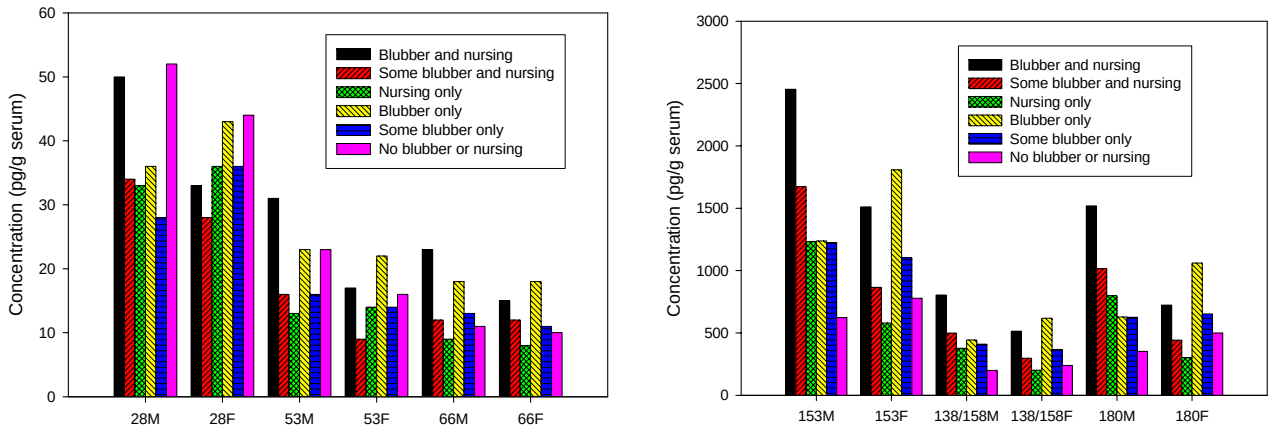

Figure 1. Concentrations of selected PCB congeners representative of low- and high-chlorination PCBs in males and females separated by blubber consumption and duration of breastfeeding. The specific congeners are displayed on the x-axis followed by an "M" for males or an "F" for females. "Blubber" indicates that blubber was consumed at both 7 and 14 years of age. "Some blubber" indicates that blubber was consumed either at 7 years or at 14 years but not both. "Nursing" indicates the child was breastfed for more than 2 months.